

Seasonal changes in diet affect fatty acid composition of Jersey milk in organic production system

Sezonske promjene sastava obroka utječu na sastav masnih kiselina mlijeka krava Jersey pasmine u ekološkom uzgoju

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ABSTRACT

The aim of this study was to determine effect of diet-based seasonal changes on fatty acid composition of Jersey milk in mountain region of Croatia. Bulk milk samples (200 mL) were collected during total-mixed ratio (TMR)-based, intermediate and pasture-based diet. Fatty acid composition of milk was determined by gas-chromatography. We found that pasture-based diet has positive effect on fatty acid composition of Jersey milk considering human nutrition. During pasture-based diet, Jersey cows produced milk with lower ($P<0.05$) saturated fatty acid and higher ($P<0.05$) polyunsaturated fatty acid content. Namely, regarding individual fatty acids, milk produced during pasture-based diet had lower ($P<0.05$) C16:0 and higher ($P<0.05$) C18:2n-6, C18:3n-6 and conjugated linoleic acid (CLA) content than milk produced during TMR and intermediate diet. Milk produced during pasture-based diet can be valuable source of health-beneficial PUFA in diet.

Keywords: dairy, pasture-based diet, total-mixed ratio, polyunsaturated fatty acids

SAŽETAK

Cilj ovog istraživanja bio je utvrditi utjecaj sezonskih promjena u sastavu obroka na profil masnih kiselina u mlijeku krava Jersey pasmine. Skupni uzorci mlijeka iz laktofriza (200 mL) prikupljeni su tijekom prehrane bazirane na potpuno izmiješanom obroku (TMR), prijelaznog razdoblja sa TMR-a na ispašu i samo ispaše. Profil masnih kiselina je određen plinskom kromatografijom. Utvrđeno je da hranidba bazirana na ispaši ima pozitivan utjecaj na profil masnih kiselina sa stanovišta ljudske prehrane. Tijekom hranidbe bazirane na ispaši u mlijeku Jersey krava utvrđen je manji ($P<0,05$) udio zasićenih masnih kiselina i veći ($P<0,05$) sadržaj polinezasićenih masnih kiselina. Naime, što se tiče udjela pojedinačnih masnih kiselina, mlijeko proizvedeno tijekom pašne sezone imalo je manji ($P<0,05$) udio C16:0 i veći ($P<0,05$) udio C18:2n-6, C18:3n-6 te CLA masnih kiselina. Mlijeko proizvedeno tijekom pašne sezone može biti vrijedan izvor višestruko nezasićenih masnih kiselina koji imaju poželjan nutritivni učinak.

Ključne riječi: mlijeko, ispaša (napasivanje), potpuno izmiješani obrok, višestruko nezasićene masne kiseline

INTRODUCTION

Proximate chemical composition of bovine milk includes approximately 12.5% of dry matter with 4.2% of milk fat (Lindmark Månsson, 2008). Lipids in milk are in form of spherical droplets i.e. milk fat globules and milk is considered as an oil-in-water emulsion. Per one millilitre of the milk there are about 10^{10} milk fat globules (Walstra et al., 1999). Up to 98% of milk fat is made of triglycerides, while the rest belongs to diacylglycerol, cholesterol, phospholipids and free fatty acids (Jensen and Newburg, 1995).

Milk contains over 400 different fatty acids, so milk fat has one of the most complex fatty acid profiles when compared with other plant or animal originating fats and oils (Sichien et al., 2009). However, majority of fatty acids in bovine milk are present in traces and about 15 of them can be found in proportion of 1% or higher (Lindmark Månsson, 2008). Short and medium milk fatty acids (C4 to C14) are synthesised *de novo* in mammary gland, while C16:0 can be either *de novo* synthesised or originate from feed (Santos, 2002; Bauman et al., 2011). Longer chain fatty acids (C16:0 and longer) originate from both feed or mobilized body fat; in milk these are dominantly C18 fatty acids (Larsen et al., 2011). Content of saturated fatty acids (SFA) in milk can be up to 75% with palmitic acid as dominant one (30%). Oleic acid is dominant monounsaturated fatty acid (MUFA) with approximately 25%, while polyunsaturated fatty acids (PUFA) make up to 5% of total fatty acid weight in milk (Lindmark Månsson, 2008). Due to rumen microorganisms, PUFAs originating from feed go under intensive biohydrogenation. It was found that biohydrogenation of two main PUFA in milk, linoleic (1.6%) and linolenic (0.7%) ranges from 70 to 95% i.e., from 85 to 100% (Doreau and Ferlay, 1994; Haug et al., 2007).

Milk fat content and fatty acid profile affect nutritional, physical and technological properties of milk (Miller et al., 2007; Sichien et al., 2009). Nutritionally, C14:0 and C16:0 are considered to have negative health effect leading to cardiovascular diseases and increased LDL cholesterol level (Haug et al., 2007). However, milk and

dairy products are also dietary sources of essential fatty acids with beneficial effects on human health (Haug et al., 2007). Milk fat content and fatty acid profile are highly depended on dairy cow's nutrition i.e., ration composition. Previous studies have reported favourable effect of pasture-based diet on milk fatty acid composition. Namely, milk produced during pasture season generally should have lower content of dominant SFA and higher content of favourable MUFA and PUFA (Elgersma et al., 2015; Kay et al., 2004; Vanhatalo et al., 2007; Gómez-Cortés et al., 2009). Fatty acid milk profile of grazing organic dairy cows is characterised by higher content of PUFA and CLA (Chilliard et al., 2007; White et al., 2001; Elgersma, 2015) compared to housed conventional cows. This is very important in human nutrition because CLA acts as an anticarcinogenic agent (Parodi, 1999).

In Croatia and regionally, Jersey breed is newer dairy breed in milk production. Jersey breed is characterized by smaller body size, easy calving and higher performance in milk yield, fat and protein content in relation to their bodyweight. Due to that, Jersey dairy cows are suitable for low-input organic farms that utilize naturally available pastures and producing high quality milk. Thus, the aim of this paper was to determine variations in fatty acid composition of Jersey milk from organic production system affected by season-based changes in diet.

MATERIAL AND METHODS

Animals, Diet and Experimental Design

The research was conducted on a Jersey organic dairy farm located in hilly - mountainous region of Croatia (Medak, Gospić, Lika -Senj County; lat. 44.4554100, long. 15.5077600; altitude 574 m). Cows were milked twice a day in automated milking parlour. Diet-based seasonal changes are present on the farm and seasonal feeding differences were as follows: TMR- based, TMR + pasture-based (intermediate) and pasture-based diet.

Components of rations during different feeding seasons are presented in Table 1. Due to farm location in hilly-mountainous region and climate characteristics of Lika region, some seasons are prolonged, and some

are shorter than other. TMR-based diet was applied from October to March and was based on alfalfa haylage. The intermediate diet was applied during early spring (April) and late summer (August and September) when the dairy cows were kept for half a day at pasture and half a day on the farm where TMR was available (Table 1). Pasture-based diet lasted from May to July, when dairy cows were kept between morning and evening milking on perennial natural pastures and only concentrate was additionally included in the diet (Table 1). The amount of pasture available before grazing during intermediate and pasture-based feeding was judged to ensure a daily pasture consumption of approximately 5 kg DM per day and 10 kg DM per day, respectively.

Table 1. Ration components of three different (season-based) diets

Ration component	Diet (kg/cow)		
	TMR	Intermediate	Pasture
Grass hay	3.0	3.0	-
Alfalfa haylage	17.0	9.0	-
Ground corn grain	4.5	3.0	6.0
Oat grain	1.28	1.35	1.5
Wheat meal	1.0	-	2.0
Wheat grain	0.85	0.85	1.5
Soybean cake	1.0	-	-
Sunflower cake	1.0	0.6	-
Molasses	1.0	0.6	-
Vitamins and minerals	0.18	0.15	0.15
Calcium carbonate	0.07	0.07	0.07

Milk sampling

Bulk milk samples were collected each month during TMR-based, intermediate and pasture-based diet. Milk produced over the last week of each month was collected in a bulk tank and sampling of the collected milk occurred at the end of the week. For each sampling ($n=12$), 200 mL of bulk tank milk was collected in sterile tubes and stored at $-20\text{ }^{\circ}\text{C}$ until the analyses. Six bulk milk samples were collected during TMR-based diet, three samples during intermediate and three samples during pasture-based diet.

Methods

Before analyses, milk samples were thawed, mixed and homogenised. In order to determine milk fatty acid profiles from cows fed different seasonal diets, milk fat was first extracted following three-step extraction with gravimetric reference method (ISO, 2010). Milk fat transesterification (ISO, 2002a) was performed in order to obtain fatty acid methyl esters (FAME). Individual fatty acids were determined by gas-liquid chromatography (ISO, 2002b).

Frozen milk samples were thawed in water bath at $38\text{ }^{\circ}\text{C}$. From each sample, part of 10 g was taken and placed in extraction tube (80 mL volume). In each tube 2 mL ammonium solution (25%), 10 mL ethanol ($\geq 99.8\%$) and 25 mL petroleum (40 – 60 $^{\circ}\text{C}$ boiling interval) were added. After adding solvents, tubes were hand shaken and left to precipitate. Then precipitate was transferred in flasks and solvents were evaporated by heating in water bath at $40\text{ }^{\circ}\text{C}$ for approximately 10 minutes. After evaporation, second-step extraction procedure was done by repeating first step and addition of 15 mL diethyl ether and 15 mL petroleum. Precipitation and evaporation were repeated under same conditions as in the first step. Third extraction step was done without ethanol and with addition of 15 mL diethyl ether and 15 mL petroleum followed by evaporation. After third extraction step, 4 mL of diethyl ether and 4 mL of petroleum was added in flasks and obtained mixture was then transferred into tubes for lipid collection. Removal of solvents was done by standard nitrogen for 30 minutes and in the end in the flask was left only lipids.

In each flask containing extracted lipid was added N-heptane (1200 μL), 10% solution of fat in n-heptane (300 μL) and 2 mol/L solution of KOH in methanol (60 μL). Samples were then vigorously shaken for 2 minutes in vortex mixer (Vortex-Genie®2, Scientific Industries, Inc., USA). Then was added 150 mg of $\text{NaHSO}_4 \times \text{H}_2\text{O}$ and samples were vortexed for 1 min and centrifuged at $1000\times g$ for 3 min (Eppendorf 5417R centrifuge, Hamburg, Germany). Obtained supernatant (1 μL) was used for gas chromatography. The gas chromatograph (Agilent 7890A;

Agilent, Santa Clara, CA, USA) was equipped with Agilent 7683B autosampler (Agilent, Santa Clara, CA, USA), split injection port (1:130), flame ionisation detector and a 60-m fused silica capillary column (0.25 mm of internal diameter) coated with a 0.20 µm film of CP-Sil 88 (Agilent, Santa Clara, CA, USA). As carrier gas was used hydrogen applied at constant flow of 1.3 mL/min. Temperature of injector and detector was 255 °C. The temperature of oven was kept at 50°C, then programmed to increase 5 °C each minute till 225 °C (3 min isothermal) and then programmed to increase 1 °C each minute till 237 °C. Chromatograms were evaluated with Agilent Software EZ Chrom Elite 3.3.2. The fatty acid calibration was done according to the reference milk fat CRM 164 (IRMM, Geel, Belgium). Thirty fatty acids were determined and calculated as weight percentage (g/100 g fatty acids).

Data were analysed in SAS V9.4 (SAS Inst. Inc., 2013). Distribution of the data was tested by Shapiro-Willkis test, and dates with normal distribution were analysed using GLM procedure, while Kruskal-Wallis test was used as non-parametric test. Significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Composition of SFA in Jersey milk during seasons with different diets is presented in Table 2. The most abundant SFA in Jersey milk was C16:0 followed by C18:0 and C14:0. More than half of all SFA were present in proportion less than 1%. Milk produced during pasture-based season had lower ($P < 0.05$) C16:0, but higher ($P < 0.05$) C18:0 and C20:0 content than milk during TMR-based and intermediate diet. Different diets had no effect on content of C14:0. Pasture-based diet resulted in lower ($P < 0.05$) content of C13:0 and C15:0 compared to TMR-based or intermediate diet. White et al. (2001) reported higher content of C14:0 in milk from pasture than TMR based feeding system, but no difference in content of C16:0 and C18:0. Similar effect of pasture-based diet on dominant SFA was also reported by Palladino et al. (2010). However, Romanzin et al. (2013) and Frelich et al. (2009) reported lower C14:0 and C16:0, but higher C18:0 content in milk originating from pasture compared to indoor system.

Table 2. Composition of saturated fatty acids in Jersey milk effected by different diets (n=12)

Saturated fatty acids in milk (g/100 g fatty acids)	Diet		
	TMR	Intermediate	Pasture
C4:0	0.83±0.10	1.03±0.15	0.79±0.15
C6:0	1.41±0.14	1.33±0.19	1.05±0.19
C8:0	0.85±0.06	0.97±0.09	0.75±0.09
C10:0	2.26±0.14	2.27±0.20	1.87±0.20
C11:0	0.15±0.08	0.20±0.10	0.03±0.11
C12:0	3.14±0.12	3.17±0.17	2.52±0.17
C13:0	0.10±0.00 ^a	0.09±0.01 ^a	0.06±0.01 ^b
C14:0	11.19±0.28	11.05±0.39	10.04±0.39
C15:0	1.34±0.06 ^a	1.11±0.08 ^a	0.96±0.07 ^b
C16:0	37.96±1.28 ^a	35.86±1.18 ^a	33.83±1.81 ^b
C17:0	0.76±0.05	0.68±0.07	0.78±0.07
C18:0	12.69±0.49 ^a	11.81±0.69 ^a	15.63±0.69 ^b
C20:0	0.21±0.01 ^a	0.21±0.01 ^a	0.24±0.01 ^b
C21:0	0.05±0.00	0.04±0.00	0.05±0.00
C22:0	0.10±0.00	0.09±0.00	0.11±0.00
C23:0	0.04±0.00	0.03±0.00	0.05±0.00
C24:0	0.05±0.01	0.19±0.07	0.06±0.07

^{a,b} values marker with different letter differ significantly ($P < 0.05$)

Observed differences between present and previous studies can be explained as result of differences in the ration composition and available sources of SFA in diet during different seasons.

Composition of MUFA in Jersey milk during different feeding systems is presented in Table 3. Dominant MUFA in Jersey milk was C18:1n-9, while other MUFA were presented in percentage around 1 or lower. Diet-based changes during different seasons resulted in lower ($P < 0.05$) C14:1 and higher ($P < 0.05$) C18:1n-7 content during pasture season. Intermediate period with combination of pasture and TMR in diet resulted in lower ($P < 0.05$) C18:1n-9 content, while no difference was observed between pasture-based and TMR-based

diet. Contrary to the present study, White et al. (2001) and Palladino et al. (2010) reported no effect of ration changes during seasons on C18:1n-9 in milk. However, Romanzin et al. (2013) and Frelich et al. (2009) reported higher C18:1n-9 content in milk originating from pasture compared to indoor season. According to Schwendel et al. (2015), most of analysed FA (43 out of 51) were influenced by season whilst linoleic, α -linolenic and CLA being more abundant in milk harvested during autumn. Breed-related differences and dietary fatty acid sources can be main causes of differences found between these studies.

Table 3. Composition of monounsaturated fatty acids in Jersey milk affected by different diets (n=12)

Monounsaturated fatty acids in milk (g/100 g fatty acids)	Diet		
	TMR	Intermediate	Pasture
C14:1	0.86±0.04 ^a	0.82±0.05 ^a	0.56±0.05 ^b
C16:1	1.39±0.14	1.38±0.19	1.30±0.19
C18:1n-9	21.37±0.89 ^a	18.47±1.26 ^b	22.76±1.26 ^a
C18:1n-7	1.36±0.21 ^a	1.45±0.30 ^a	2.99±0.30 ^b
C20:1	0.03±0.00	0.03±0.00	0.04±0.00

^{a,b} values marker with different letter differ significantly ($P<0.05$)

Composition of polyunsaturated fatty acids in Jersey milk during different rearing systems is presented in Table 4. Beneficial effect of grazing on PUFA content in Jersey milk was found. The content of C18:2n-6, C18:3n-6 and CLA was higher ($P<0.05$) during pasture season than during indoor TMR based diet. Significantly higher content of these PUFA in milk was also reported by Romanzin et al. (2013) and White et al. (2001), while Palladino et al. (2010) reported significant effect of season on C18:2n-6, but not on CLA. Higher C18:2n-6 and CLA content during pasture season than indoor was also reported by Frelich et al. (2009). Observed differences between mentioned studies can be attributed mainly to differences in diet and ration composition.

Sums and ratios of fatty acids in Jersey milk during different rearing regimes are presented in Table 5. During grazing period milk had lower ($P<0.05$) total content of

SFA and higher ($P<0.05$) total content of PUFA n-6 than milk during TMR-based and intermediate diet. Nutritional value of dietary food is most often evaluated through fatty acid ratios, like PUFA/SFA and n-6/n-3.

Table 4. Composition of polyunsaturated fatty acids in Jersey milk affected by different diets (n=12)

Polyunsaturated fatty acids in milk (g/100 g fatty acids)	Diet		
	TMR	Intermediate	Pasture
C18:2n-6	1.72±0.06 ^a	1.79±0.09 ^a	1.91±0.09 ^b
C18:3n-6	0.02±0.00 ^a	0.01±0.00 ^a	0.13±0.00 ^b
C18:3n-3	0.51±0.06	0.54±0.09	0.63±0.09
CLA	0.40±0.04 ^a	0.42±0.05 ^a	0.57±0.05 ^b
C20:3n-6	0.10±0.00	0.09±0.00	0.09±0.00
C20:4n-6	0.16±0.00	0.17±0.00	0.16±0.00
C20:5n-3	0.05±0.00	0.05±0.00	0.05±0.00
C22:5n-3	0.09±0.00	0.10±0.00	0.10±0.00

^{a,b} values marker with different letter differ significantly ($P<0.05$)

Table 5. Fatty acid sums and ratios of Jersey milk affected by different diets

Fatty acid sums and ratios	Diet		
	TMR	Intermediate	Pasture
SFA	72.85±1.71 ^a	70.14±2.42 ^{ab}	68.82±2.42 ^b
MUFA	25.01±0.83	22.15±1.19	27.65±1.19
PUFA	3.05±0.14 ^a	3.17±0.19 ^{ab}	3.53±0.19 ^b
PUFAn-6	2.40±0.90	2.49±0.12	2.75±0.12
PUFAn-3	0.65±0.07	0.69±0.09	0.79±0.09
PUFA/SFA	0.05±0.00	0.05±0.00	0.05±0.00
n-6/n-3	3.79±0.26	3.80±0.37	3.53±0.37

^{a,b} values marker with different letter differ significantly ($P<0.05$); PUFA n-6 - sum of n-6 polyunsaturated fatty acids, PUFA n-3 - sum of n-3 polyunsaturated fatty acids, PUFA/SFA - ratio of polyunsaturated and saturated fatty acids, n-6/n-3 - ratio of n-6 and n-3 polyunsaturated fatty acids

Generally, it is recommended that PUFA/SFA ratio should be >0.4 and n-6/n-3 <4.0 . For dairy products, PUFA/SFA ratio can range from 0.02 to 0.18 (Chen and Liu, 2020) and in the present study was less favourable than recommended. Rego et al. (2016) reported PUFA/

SFA ratio in Holstein milk similar to that found in this study. Lower value of n-6/n-3 ratio in milk than found in this study was reported by Romanzin et al. (2013) and Palladino et al. (2010), while Morales et al. (2015) reported similar values.

CONCLUSION

Pasture-based diet has beneficial effect on Jersey milk fatty acid composition, especially reflected in lower content of SFA and higher content of essential PUFA, like C18:2n-6, and CLA. Grazing on perennial mountain pastures in general contributes to improved fatty acid profile in Jersey milk and further research should be done to determine weekly and monthly changes in milk fatty acid composition during pasture season depending on other factors, like parity and lactation stage.

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